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Stimulated renal tubular epithelial cells induce anergy in CD4$^+$ T cells

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Stimulated renal tubular epithelial cells induce anergy in CD4$^+$ T cells. Renal tubular epithelial cells (TEC) can express MHC class II molecules *in vitro* and *in vivo*. Their ability to also secrete cytokines and express adhesion molecules suggests a possible immune accessory role for TEC. We have previously documented that TEC process and present antigen to T cell hybridomas. However, engagement of the T cell receptor alone is sufficient to induce IL-2 secretion by T cell hybridomas. We now report that presentation of antigen by TEC to a CD4$^+$ T cell clone results in functional inactivation of the T cells. Despite antigen-specific anergy, these T cells are viable and proliferate in response to IL-2. Furthermore, allogeneic antigen presenting cells were unable to restore the T cell proliferative response, suggesting that the mechanism(s) was not entirely costimulator-dependent.

Renal tubular epithelial cells (TEC) comprise the majority of the kidney cortex. The primary physiologic function of TEC is to maintain salt and water homeostasis. However, recent evidence suggests that these cells may have an immune accessory role. Notably TEC can be induced to express major histocompatibility complex class II (Ia) products during allograft rejection, autoimmune and ischemic renal injury, and graft versus host disease [1–5]. Furthermore, we and others established that in pathologic situations, TEC express the accessory molecules intercellular adhesion molecule-1 (ICAM-1) [6, 7] and vascular cell adhesion molecule-1 (VCAM-1) [8], and produce cytokines, including tumor necrosis factor α [9], interleukin-8 [10], interleukin-6 (unpublished observations), and transforming growth factor β [11].

The role of Ia on parenchymal cells has been the subject of much investigation. It has been proposed that "aberrant" expression of MHC class II molecules can result in the presentation of self antigens to T cells, with the subsequent development of organ-specific autoimmune disease [12, 13]. However, using a transgenic kidney transplant model, we have recently shown that Ia expression by TEC alone is insufficient to induce immune renal injury (**Note added in proof**). An important function of Ia-positive hematopoietic cells is that of antigen presentation. Dendritic cells, B lymphocytes and macrophages process and present exogenous antigen to CD4$^+$ T cells. T helper (Th) cells appear to require two signals in order to become activated. The first signal is engagement of the T cell receptor by the peptide-MHC complex. Signal 2, also referred to as the costimulatory signal, can be provided by certain cytokines and adhesion molecules [14]. Jenkins and Schwartz have shown that TCR engagement by cells incapable of providing a costimulatory signal results in a state of antigen-specific unresponsiveness known as anergy [15, 16]. Th cells rendered anergic fail to respond to antigen-presenting cells expressing signals 1 and 2, but remain viable, proliferating in response to IL-2.

To further evaluate the ability of TEC to stimulate T cells, we investigated the interaction between T cells and TEC in an *in vitro* system. We now report that although TEC stimulate IL-2 production by antigen-specific, MHC-restricted T cell hybridomas, they induce a state of anergy in CD4$^+$ T cell clones. The induction of anergy by TEC appears to be independent of costimulation. Although TEC express many of the molecules associated with the induction of immune responses, our results indicate that under certain conditions TEC may down-regulate autoimmune responses within the kidney.

Methods

Mice

C3H/FeJ (H-2^k) and BALB/c (H-2^d) mice were obtained from The Jackson Laboratory (Bar Harbor, Maine, USA) and maintained in our animal facility on standard lab chow. Mice were used between 6 and 12 weeks of age.

T cell clones and hybrids

A.E7 is a pigeon cytochrome c-specific, I-E^k-restricted Th1 clone [17]. A2.A2 is a subclone of the T cell hybridoma A2.2B2 which recognizes hen egg lysozyme in the context of I-A^k [18]. A.E7 and A2.A2 were provided by Drs. Abul Abbas (Harvard Medical School, Boston, Massachusetts, USA) and Laurie Glimcher (Harvard School of Public Health, Boston, Massachusetts, USA), respectively.

TEC clone

CS3.1 is a renal tubular epithelial cell clone derived from C3H/FeJ mice. The method of isolation has been previously

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described [19]. Briefly, TEC were prepared by collagenase dispersion and sequential sieving of renal cortices. Cells were subcloned by the limiting dilution technique after transformation with origin-defective SV40 DNA. Characterization of the tubular nature of these cells was as follows. CS3.1 cells display epithelial morphology and express the brush border enzyme alkaline phosphatase. They stain positively for cytokeratin and vimentin. They express ICAM-1 constitutively, and IFN-γ up-regulates ICAM-1 and induces Ia expression.

Reagents

Tissue culture media and reagents were purchased from Gibco (Grand Island, New York, USA). Mitomycin c, pigeon cytochrome c (PCC), and hen egg lysozyme (HEL) were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA). Monoclonal antibodies (mAb) against I-E^k (clone 14.4.4S, American Type Culture Collection, Rockville, Maryland, USA) and the murine CD3-ϵ chain (clone 145.2C11 provided by Dr. Jeffrey Bluestone, University of Chicago, Chicago, Illinois, USA) were obtained by affinity purification of supernatants over protein A-Sepharose CL-4B columns (Pharmacia, Piscataway, New Jersey, USA). Murine rIFN-γ was a gift from Genentech (San Francisco, California, USA).

T cell clone proliferation assay

All cultures were performed in RPMI 1640 supplemented with 10% fetal bovine serum (FBS; HyClone, Logan, Utah, USA), 50 μM 2-ME, 10 mM HEPES, 1 mM Na pyruvate, 0.1 mM non-essential amino acids, and antibiotics. Two to three $\times$ 10^4 cloned T cells were cultured in 96-well flat-bottom microtiter plates (Falcon, Lincoln Park, New Jersey, USA) in 0.2 ml of medium with one of two types of stimulator cells: 5×10^5 irradiated (1500R) syngeneic splenocytes or 2×10^3 to 5×10^4 mitomycin c-treated, IFN-γ-stimulated (100 U/ml $\times$ 72 hr) CS3.1 cells. Varying doses of antigen were used. After three days of culture, wells were pulsed with 1 μCi of [^{3}H]TdR (6.7 mCi/mM, New England Nuclear, Boston, Massachusetts, USA) and harvested six hours later using a PHD cell harvester (Cambridge Technology Inc., Cambridge, Massachusetts, USA). Thymidine incorporation was quantitated by liquid scintillation counting. Determinations were performed in triplicate. Background proliferation (without antigen) was always <1,000 cpm.

IL-2 assay

IL-2 was assayed by measuring the ability of 24-hour culture supernatants to support the growth of 1×10^4 HT-2 cells, an IL-2-dependent cell line provided by Dr. Abul Abbas. After 24 hours, cultures were pulsed with 1 μCi of [^{3}H]TdR, harvested six hours later and thymidine incorporation quantitated as above. As a positive control, HT-2 cells were also cultured with serial dilutions of human rIL-2 (gift of Dr. Jack Murphy, Boston University, Boston, Massachusetts, USA).

Fluorescence cytometry

Subconfluent monolayers of TEC were cultured for 72 hours in modified K1 medium [1:1 mixture of Dulbecco's modified Eagle's medium (DMEM) and Ham's F12, containing additives as described including 25 ng/ml of epidermal growth factor] with or without IFN-γ (100 U/ml). Cells were harvested by trypsinization, and resuspended at 5×10^5 to 1×10^6 cells/ml in PBS with 3% FBS/0.1% NaN_3. Cells were analyzed for the surface expression of B7 using the fluorescein-conjugated anti-murine B7 mAb 1G10 on a Coulter Epics Flow Cytometer. Fluorescein-conjugated anti-murine B7 mAb 1G10 on a Coulter Epics Flow Cytometer. Fluorescein-conjugated IgG2a was used as a negative control. Staining for I-E^k was performed by incubating cells with the mAb 14.4.4S, followed by FITC-conjugated anti-mouse IgG.

Table 1. Flow cytometric analysis of MHC class II expression by TEC

	TEC	Preincubation	% I-E expression
Exp. 1	CS3.1(5×10^5/well)	medium	2.0
		100 U/ml IFN-γ	95.6
Exp. 2	CS3.1(5×10^5/well)	medium	1.7
		100 U/ml IFN-γ	68.6

CS3.1 cells were cultured for 72 hours under the above conditions, and harvested by trypsinization. I-E^k expression was analyzed as described in the **Methods.**

Isolation of peritoneal exudate cells

Peritoneal exudate cells (PEC) were harvested by peritoneal lavage with 5 ml of HBSS supplemented with antibiotics. The cells were washed and resuspended in RPMI 1640 supplemented with 10% FBS and 100 μg/ml of both penicillin and streptomycin, and 10^6 cells/ml were plated on 100 mm plastic petri dishes (Fisher Scientific Co., Pittsburgh, Pennsylvania, USA). After overnight incubation at 37°C, adherent cells were washed off and resuspended in RPMI 1640 supplemented as above prior to co-culture with T cells.

Induction of T cell anergy

A.E7 cells (7×10^5/well) were preincubated in 2.0 ml medium in 24-well plates with 1×10^6 mitomycin c-treated, IFN-γ-stimulated CS3.1, with or without antigen. The T cell clones were also cultured with 5×10^6 irradiated (1500R) syngeneic splenocytes and Ag or on anti-CD3 mAb-coated wells as controls. After 24 to 48 hours of culture, T cells were recovered on Ficoll-Hypaque (Cedarlane Laboratory Ltd., Hornby, Ontario, Canada) density gradients and rested for 48 to 72 hours prior to culture for three days in 96-well microtiter plates (2 to 3×10^4 cells/well) with 5×10^5 irradiated syngeneic splenocytes and antigen or with IL-2 alone. Proliferation and IL-2 production were assayed as described above.

Results

The TEC clone CS3.1 is clearly tubular in origin and was extensively characterized as previous cell lines have been described [19]. These cells do not express MHC class II molecules constitutively. However, Ia expression on CS3.1 is readily induced by IFN-γ (Table 1). The level of Ia expression by CS3.1 is higher than previous cell lines from C3H/FeJ mice (C1, C1.1) [19]. Expression of MHC class II products is a feature common to antigen presenting cells such as B lymphocytes, macrophages and dendritic cells.

In order to determine the ability of TEC to process and present antigen, CS3.1 were cultured with the I-A^k-restricted, HEL-specific T cell hybridoma A2.A2 in the presence of

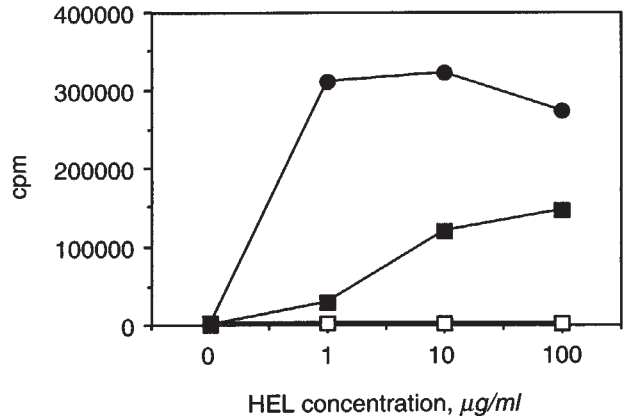

Fig. 1. *TEC stimulate IL-2 production by a T cell hybrid.* CS3.1 cells were harvested after culture in medium alone (-□-) or IFN-γ (100 U/ml) (-■-) for 72 hours. TEC (5×10^4/well) were added to 96-well culture plates containing the T cell hybridoma A2.A2 (1×10^5/well) and varying concentrations of the antigen HEL in a final volume of 0.2 ml. A2.A2 were also cultured with C3H/FeJ splenocytes (-●-) and Ag. After 24 hours, 0.05 ml of supernatant was added to the IL-2-dependent cell line HT-2 (1×10^4/well) in a final volume of 0.1 ml. After 24 hours, the wells were pulsed with [^{3}H] TdR (1 μCi/well), and IL-2 production quantitated as [^{3}H] TdR incorporation. The data shown are cpm and representative of three separate experiments. SEM for cpm values were routinely less than 20%.

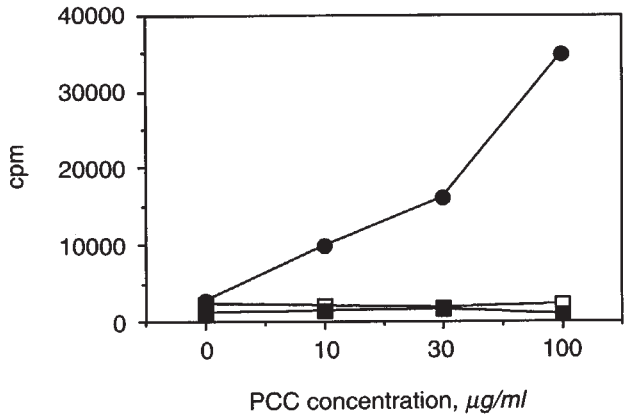

Fig. 2. *TEC fail to stimulate proliferation by a normal T cell clone.* 5×10^4 mitomycin c-treated CS3.1 (-□-) or IFN-γ-stimulated CS3.1 (-■-), or 5×10^5 1500 rad irradiated C3H/FeJ splenocytes (-●-) were cultured with 3×10^4 A.E7 cloned T cells and varying amounts of pigeon cytochrome c (PCC). Cultures were pulsed after 72 hours and harvested 6 hours later. Values are cpm and representative of three experiments. SEM were always less than 20%.

varying amounts of antigen. After 24 hours, supernatants were assayed for IL-2 using the indicator cell line HT-2. As shown in Figure 1, TEC treated with IFN-γ were able to stimulate IL-2 production by the T cell hybridoma. This response was dependent on the presence of Ag and Ia expression, since untreated TEC failed to induce IL-2 production. Peak responses to stimulated TEC, seen at HEL concentrations of 10 μg/ml were 2.5- to 3-fold lower than those to spleen cells.

T cell hybridomas often do not require costimulatory ligands, and can be stimulated by Ia molecules on a planar lipid membrane [20]. To further assess the antigen presenting capacity of TEC, CS3.1 were co-cultured with a normal T cell clone, A.E7. This clone is $CD4^+$ and is of the Th1 subtype, proliferating and secreting IL-2 and IFN-γ in response to the c-terminal fragment (residues 81-104) of pigeon cytochrome c bound to $I\text{-}E^k$. In multiple experiments, A.E7 clones proliferated when cultured with syngeneic spleen cells and antigen, but did not respond to unstimulated or IFN-γ-treated CS3.1 (Fig. 2).

It has previously been shown that engagement of the T cell receptor without provision of an appropriate costimulatory signal results in antigen-specific unresponsiveness, or anergy, in $CD4^+$ T cells of the Th1 subtype [15, 16, 21]. Anergic T cells fail to secrete IL-2 or proliferate when restimulated with competent APC and Ag. The cells remain viable as manifested by their proliferative response to IL-2. In order to demonstrate that TEC could functionally inactivate T cell clones, A.E7 were cultured with CS3.1 overnight, recovered and rested for two days in medium alone, and restimulated with either spleen APC and Ag or IL-2. A representative experiment shown in Figure 3 demonstrates that A.E7 preincubated with stimulated CS3.1 and Ag no longer proliferated in response to spleen APC and specific Ag. The cells remained viable as established by their response to IL-2. This effect was also seen in T cells cultured on plate-bound anti-CD3 mAb prior to restimulation. T cell clones pre-cultured with either spleen APC and Ag, or stimulated CS3.1 without antigen were not rendered unresponsive.

Previous experiments have shown that allogeneic spleen cells can provide the necessary costimulatory signal(s) to prevent T cell unresponsiveness induced by chemically modified APC [22]. We postulated that TEC may lack the ability to deliver a second signal, and thereby functionally inactivate T cells. The B cell activation marker B7 has been shown to costimulate T cells by binding to its ligands CD28 and CTLA-4 [23–27]. CS3.1 did not stain for B7 by flow cytometry regardless of whether or not the cells had been pre-treated with IFN-γ (Fig. 4). To further define the role of costimulators in anergy induction by TEC, Th1 clones were incubated with stimulated CS3.1 and Ag. Allogeneic spleen cells or peritoneal exudate cells (PEC) were added to the cultures, but failed to restore a T cell proliferative response (Fig. 5).

Discussion

TEC express multiple molecules which would enable them to function as immune accessory cells. Notably, they can be induced to express MHC class II products *in vitro* and *in vivo* [1, 2, 4, 28, 29]. They also secrete a variety of cytokines and express the surface molecules ICAM-1 and VCAM-1. Using the transformed TEC clone CS3.1, we reported that TEC, when stimulated by IFN-γ to express Ia, can process and present protein antigen to T cell hybridomas. As we have previously noted, peak proliferative responses to TEC were almost threefold lower than those to spleen APC. A number of factors may contribute to this apparent inefficiency in antigen presentation by TEC: (i) Ia expression on TEC may be at a lower density than that on spleen cells; (ii) TEC form a monolayer, and therefore only a fraction of the cell surface is exposed to the T cells in culture; and (iii) we used tenfold fewer TEC per well than spleen cells due to steric constraints. Correcting for these variables, TEC may in fact be equally efficient at stimulating T cell hybridomas as spleen APC.

It is known that T cell hybridomas can be readily induced to

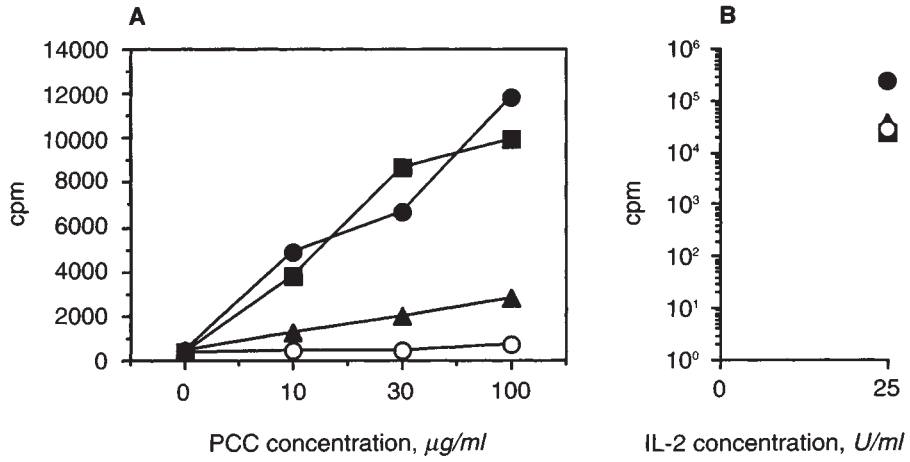

Fig. 3. *TEC induce T cell unresponsiveness* in vitro. Symbols are: (▲) TEC* + Ag; (■) TEC; (○) anti-CD3; (●) SPL + Ag. A.E7 cloned T cells (7×10^5) were incubated in 24-well plates with 1×10^6 mitomycin c-treated, IFN-γ-stimulated CS3.1 (TEC*) in the presence or absence of pigeon cytochrome c (PCC) (100 μg/ml), or 5×10^6 1500 rad irradiated C3H/FeJ splenocytes (SPL) and PCC. A.E7 were also cultured on anti-CD3 (3 μg/ml) mAb-coated wells. T cells were recovered after 24 hours, and rested in medium. After 48 hours, A.E7 cloned T cells (2×10^4/well) were restimulated in 96-well plates with 5×10^5 1500 rad irradiated C3H/FeJ splenocytes and varying concentrations of antigen (PCC), or with IL-2 (25 U/ml). After 72 hours, cultures were pulsed (1 μCi/well [^{3}H] TdR) and harvested 6 hours later. Values are cpm and representative of three experiments. SEM were less than 20%.

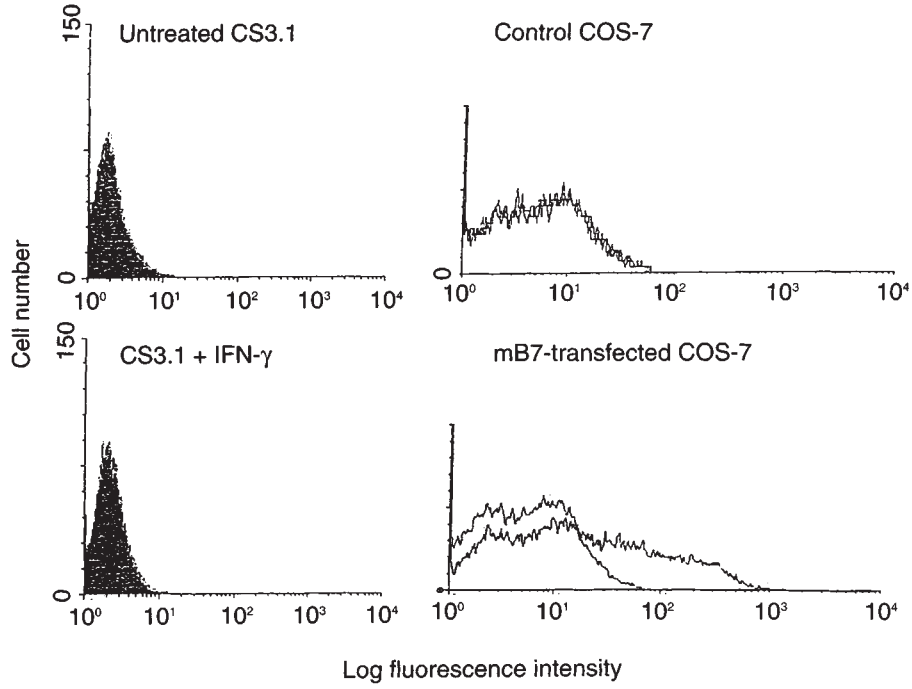

Fig. 4. *TEC do not express the costimulatory molecule B7.* CS3.1 were harvested after a 72 hour incubation in medium or IFN-γ (100 U/ml), and stained for murine B7 (mB7) with the FITC-conjugated mAb 1G10 as described in **Methods.** COS-7 and mB7-transfected COS-7 cells were used as negative and positive controls, respectively.

secrete IL-2 in response to MHC class II plus peptide on planar lipid membranes [20]. T cell clones, on the other hand, require a costimulatory signal as well as T cell receptor engagement in order to become activated [16]. Failure to provide this second signal results in antigen-specific T cell unresponsiveness, or anergy. We have shown that Ia-bearing TEC, in the presence of antigen, render the Th1 clone A.E7 unresponsive to restimulation with competent APC and antigen. The anergic T cells remain viable, proliferating in response to IL-2. Furthermore, stimulated TEC do not express the costimulatory molecule B7. Based on these results and previous studies examining the antigen presenting capacity of non-hematopoietic cells, we hypothesized that TEC may lack the necessary second signal(s) required to activate T cells. We were surprised to find that allogeneic APC (spleen cells or macrophages) failed to support T cell proliferation in response to stimulated TEC and Ag. B7 binds one of two ligands on T cells, CD28 or CTLA-4 [25, 30]. Preliminary experiments show that the mAb to murine CD28 is also unable to prevent T cell anergy induced by stimulated TEC (unpublished observations). These studies suggest that the absence of appropriate costimulatory ligands on TEC does not completely explain our observations. There exist at least two systems for inducing T cell anergy *in vitro*. T cells cultured with chemically modified APC and antigen are rendered unresponsive [15]; this state can be prevented by providing the appropriate second signal(s) on allogeneic spleen cells [22]. T cells cultured on anti-CD3 mAb-coated wells are also inactivated, but this anergic state is less costimulator-dependent, that is, allogeneic APC will not completely prevent anti-CD3 mAb-induced anergy [31]. Therefore, it could be that stimulated TEC provide the same, or a similar, signal as anti-CD3 mAb, rendering T cells unresponsive. The inhibitory signal provided

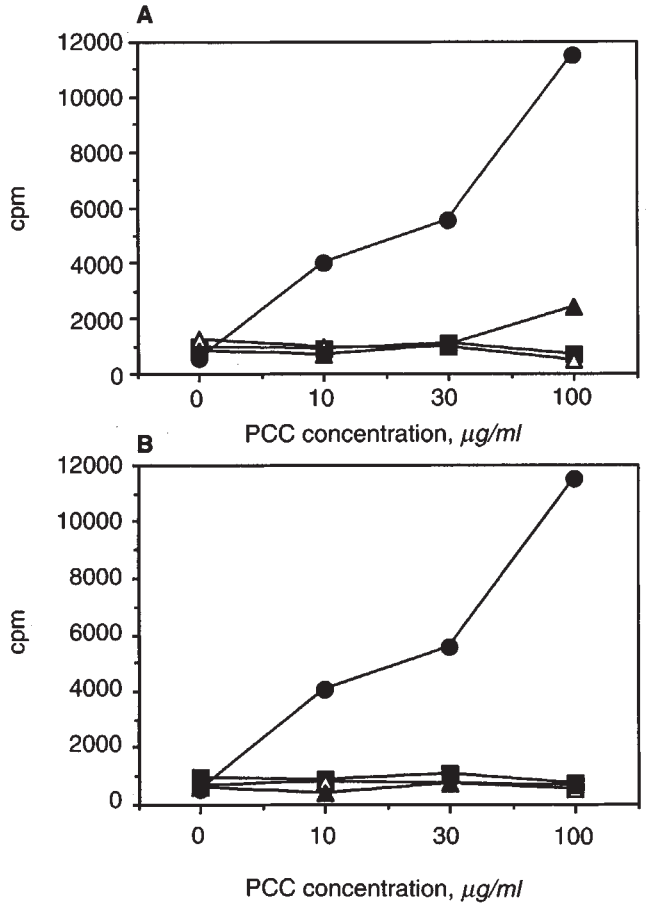

Fig. 5. *Allogeneic accessory cells fail to restore T cell proliferation in response to TEC.* A.E7 cells (3×10^4/well) were cultured with mitomycin c-treated IFN-γ-stimulated CS3.1 (TEC*) (5×10^4/well) with or without 1500 rad irradiated BALB/c splenocytes (alloSPL) (5×10^4-5×10^5/well), with or without mitomycin c-treated BALB/c peritoneal exudate cells (alloPEC) (4×10^3-4×10^4/well) and varying amounts of antigen (PCC). A.E7 T cells were also cultured with 5×10^5 1500 rad irradiated C3H/FeJ splenocytes (SPL) and antigen. Proliferation was measured after 72 hours by [^{3}H] TdR incorporation. Symbols in **A** are: (■) TEC*; (△) TEC* + alloSPL ($5 \times$ 10E4); (▲) TEC* + alloSPL ($5 \times$ 10E5); (●) SPL. Symbols in **B** are: (■) TEC*; (△) TEC* + alloPEC ($4 \times$ 10E3); (▲) TEC* + alloPEC ($4 \times$ 10E4); (●) SPL.

by TEC may result directly from engagement of the TCR-CD3 complex, or could be secondary to TEC secretion of cytokines such as transforming growth factor β.

The tubular epithelial cell line MCT, described by Haverty and colleagues, has previously been shown to stimulate the proliferation of tubular antigen (3M-1)-specific, class II MHC-restricted T cells *in vitro*, despite low-level Ia expression [32]. Therefore, cultured TEC may either stimulate or neutralize T cell clones, depending on the nature of the costimulatory signal(s) provided. A recent report by Hagerty and Allen demonstrates that TEC are capable of processing foreign and self antigen *in vivo* as manifested by their ability to stimulate T cell hybridomas *in vitro* [33]. However, there is little evidence that TEC can activate T cells *in vivo*. Further experiments are required to determine whether or not TEC can be induced to express the appropriate costimulatory molecule(s) *in vivo*. Our results indicate that, under certain conditions, TEC can functionally inactivate $CD4^+$ T cells. Therefore, TEC stimulated to express certain immune accessory molecules may function to regulate autoimmune processes within the kidney by paralyzing self-reactive T cell clones.

Acknowledgments

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Note added in proof

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References

1. WUTHRICH RP, YUI MA, MAZOUJIAN G, NABAVI N, GLIMCHER LH, KELLEY VE: Enhanced MHC class II expression in renal proximal tubules precedes loss of renal function in MRL/lpr mice with lupus nephritis. *Am J Pathol* 134:45–51, 1989
2. MÜLLER CA, MARKOVIC-LIPKOVSKI J, RISLER T, BOHME A, MÜLLER GA: Expression of HLA-DQ, DR and DP antigens in normal kidney and glomerulonephritis. *Kidney Int* 35:116–124, 1989
3. SINCLAIR GD, WADGYMAR A, HALLORAN PF, DELOVITCH TL: Graft-vs-Host reactions induce H-2 class II gene transcription in host kidney cells. *Immunogenetics* 20:503–511, 1984
4. BENSON EM, COLVIN RB, RUSSELL PS: Induction of Ia antigens in murine renal transplants. *J Immunol* 134:7–9, 1985
5. SHOSKES DA, PARFREY NA, HALLORAN PF: Increased major histocompatability complex antigen expression in unilateral ischemic acute tubular necrosis in the mouse. *Transplantation* 49:201–207, 1990
6. WUTHRICH RP, JEVNIKAR AM, TAKEI F, GLIMCHER LH, KELLEY VE: Intercellular adhesion molecule-1 (ICAM-1) is upregulated in autoimmune lupus nephritis. *Am J Pathol* 136:441–450, 1990
7. BISHOP GA, HALL BM: Expression of leukocyte and lymphocyte adhesion molecules in the human kidney. *Kidney Int* 36:1078–1085, 1989
8. WUTHRICH RP, JENKINS TA, SNYDER TS: Regulation of cytokine-stimulated vascular cell adhesion molecule-1 expression in renal tubular epithelial cells. *Transplantation* 55:172, 1993
9. JEVNIKAR AM, BRENNAN DC, SINGER GG, HENG JE, MASLINSKI W, WUTHRICH RP, GLIMCHER LH, RUBIN-KELLEY VE: Stimulated kidney tubular epithelial cells express membrane associated and secreted TNFα. *Kidney Int* 40:203–211, 1991
10. SCHMOUDER RL, STRIETER RM, WIGGINS RC, CHENSUE SW, KUNKEL SL: *In vitro* and *in vivo* interleukin-8 production in human renal cortical epithelia. *Kidney Int* 41:191–198, 1992
11. THOMPSON NL, FLANDERS KC, SMITH JM, ELLINGSWORTH LR, ROBERTS AB, SPORN MB: Expression of transforming growth factor-β1 in specific cells and tissues of adult and neonatal mice. *J Cell Biol* 108:661–669, 1989
12. PUJOL-BORRELL R, TODD I, LONDEI M, FOULIS A, FELDMANN M, BOTTAZZO GF: Inappropriate major histocompatibility complex class II expression by thyroid follicular cells in thyroid autoimmune disease and by pancreatic beta cells in type I diabetes. *Mol Biol Med* 3:159–165, 1986
13. BOTTAZZO GF, PUJOL-BORRELL R, HANAFUSA T, FELDMANN M: Role of aberrant HLA-DR expression and antigen presentation in the induction of endocrine autoimmunity. *Lancet* 2:1115–1118, 1983

14. JENKINS MK: The role of cell division in the induction of clonal anergy. *Immunol Today* 13:69–73, 1992
15. JENKINS MK, SCHWARTZ RH: Antigen presentation by chemically modified splenocytes induces antigen-specific T cell unresponsiveness *in vitro* and *in vivo*. *J Exp Med* 165:302–319, 1987
16. SCHWARTZ RH: A cell culture model for T lymphocyte clonal anergy. *Science* 248:1349–1356, 1990
17. HECHT TT, LONGO DL, MATIS LA: The relationship between immune interferon production and proliferation by antigen-specific, MHC-restricted T cell lines and clones. *J Immunol* 131:1049, 1983
18. ALLEN PM, MCKEAN DJ, BECK BN, SHEFFIELD J, GLIMCHER LH: Direct evidence that a class II molecule and a simple globular protein generate multiple determinants. *J Exp Med* 162:1264–1274, 1985
19. WUTHRICH RP, GLIMCHER LH, YUI MA, JEVNIKAR AM, DUMAS SE, KELLEY VE: MHC class II, antigen presentation and tumor necrosis factor in renal tubular epithelial cells. *Kidney Int* 37:783–792, 1990
20. QUILL H, SCHWARTZ RH: Stimulation of normal inducer T cell clones with antigen presented by purified Ia molecules in planar lipid membranes: Specific induction of a long-lived state of proliferative nonresponsiveness. *J Immunol* 138:3704–3712, 1987
21. MUELLER DL, JENKINS MK, SCHWARTZ RH: Clonal expansion versus functional clonal inactivation: A costimulatory signalling pathway determines the outcome of T cell antigen receptor occupancy. *Annu Rev Immunol* 7:445–480, 1989
22. JENKINS MK, ASHWELL JD, SCHWARTZ RH: Allogeneic non-T spleen cells restore the responsiveness of normal T cell clones stimulated with antigen and chemically modified antigen-presenting cells. *J Immunol* 140:3324–3330, 1988
23. HARDING FA, MCARTHUR JG, GROSS JA, RAULET DH, ALLISON JP: CD28-mediated signalling co-stimulates murine T cells and prevents induction of anergy in T-cell clones. *Nature* 356:607–609, 1992
24. REISER H, FREEMAN GJ, RAZI-WOLF Z, GIMMI CD, BENACERRAF B, NADLER LM: Murine B7 antigen provides an efficient costimulatory signal for activation of murine T lymphocytes via the T-cell receptor/CD3 complex. *Proc Natl Acad Sci USA* 89:271–275, 1992
25. LINSLEY PS, BRADY W, URNES M, GROSMAIRE LS, DAMLE NK, LEDBETTER JA: CTLA-4 is a second receptor for the B cell activation antigen B7. *J Exp Med* 174:561–569, 1991
26. LINSLEY PS, BRADY W, GROSMAIRE L, ARUFFO A, DAMLE NK, LEDBETTER JA: Binding of the B cell activation antigen B7 to CD28 costimulates T cell proliferation and interleukin 2 mRNA accumulation. *J Exp Med* 173:721–730, 1991
27. JENKINS MK, TAYLOR PS, NORTON SD, URDAHL KB: CD28 delivers a costimulatory signal involved in antigen-specific IL-2 production by human T cells. *J Immunol* 147:2461–2466, 1991
28. BISHOP GA, WAUGH JA, HALL BM: Expression of HLA antigens on renal tubular cells in culture. II. Effect of increased HLA antigen expression on tubular cell stimulation of lymphocyte activation and on their vulnerability to cell-mediated lysis. *Transplantation* 46: 303–310, 1988
29. BISHOP GA, HALL BM, SURANYI MG, TILLER DJ, HORVATH JS, DUGGIN GG: Expression of HLA antigens on renal tubular cells in culture. I. Evidence that mixed lymphocyte culture supernatants and gamma interferon increase both class I and class II HLA antigens. *Transplantation* 42:671–679, 1986
30. GROSS JA, ST. JOHN T, ALLISON JP: The murine homologue of the T lymphocyte antigen CD28: Molecular cloning and cell surface expression. *J Immunol* 144:3201–3210, 1990
31. WILLIAMS ME, LICHTMAN AH, ABBAS AK: Anti-CD3 antibody induces unresponsiveness to IL-2 in Th1 clones but not in Th2 clones. *J Immunol* 144:1208–1214, 1990
32. HAVERTY TP, KELLY CJ, HINES WH, AMENTA PS, WATANABE M, HARPER RA, KEFALIDES NA, NEILSON EG: Characterization of a renal tubular epithelial cell line which secretes the autologous target antigen of autoimmune experimental interstitial nephritis. *J Cell Biol* 107:1359–1368, 1988
33. HAGERTY DT, ALLEN PM: Processing and presentation of self and foreign antigens by the renal proximal tubule. *J Immunol* 148:2324–2330, 1992